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**Patentanmeldung Nr.    Patent application No.    Demande de brevet n°**

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Im Auftrag

For the President of the European Patent Office

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Enzymatic modification of triglyceride fats

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## Enzymatic modification of triglyceride fats

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### 10 Field of the invention

The present invention deals with an interesterification process of triglyceride fats. More particularly the process concerns an enzymatic interesterification process which is  
15 further denoted as an enzymatic rearrangement process.

Chemical interesterification of a triglyceride fat aims at an exchange of the fatty acid residues connected to the glyceride moiety of the fat. In the resulting triglycerides  
20 fatty acid residues have been exchanged by other residues, originating from the same or different glyceride molecules or from free fatty acids when present in the reaction mixture.

25 Such chemical interesterification process needs a catalyst, which usually is an alkali metal hydroxide or an alkali metal alkanolate, such as sodium methanolate. The exchange of fatty acid residues eventually results in a statistically random distribution of the fatty acid residues over the  
30 terminal and middle positions of the glyceride molecule. The obtained fat is said to have become fully randomised.



Since consumers increasingly prefer food and food ingredients which have not been exposed to a chemical treatment, a general need has arisen for non-chemical modification processes of triglyceride fats. Chemical

5 interesterification may be substituted by an enzymatic rearrangement process. Such process does not affect the naturalness of the starting fat.

For enzymatic rearrangement (ER) a lipase enzyme is used as  
10 catalyst. Lipases used for ER comprise the microbial *Mucor miehei* lipase and *Thermomyces lanuginosa* lipase. These lipases are 1,3-specific which means that they only affect fatty acid residues on the terminal positions of the glyceride moiety of the fat molecule. The ester bond on the  
15 middle position remains unaffected, with the result that the rearrangement process is limited to the terminal positions. *Thermomyces lanuginosa* lipase immobilized as an aggregate with silica is a food grade catalyst and available on industrial scale under the name Lipozyme TL IM™.

20

WO96/14756 describes ER of fat blends using 1,3-specific SP392 as lipase catalyst. The process is characterized in that the rearrangement does not proceed beyond a conversion degree of 90% (but being at least 20%).

25

An ER process which affects only the terminal fatty acid residues delivers triglyceride products with a triglyceride composition which is quite different from the fully randomised triglyceride fat resulting from chemical  
30 interesterification. A first adverse consequence is that the extensive knowledge and experience built for the use of fully randomised chemically interesterified fats in the manufacture of food products can not be used. Another

adverse consequence is that 1,3-specific ER is not able to increase the amount of triglycerides with a saturated middle position which position usually is unsaturated in a natural feedstock fat.

5

Attempts have been made to modify the ER process such that also the middle position is randomized.

Some rare lipases including *Candida cylindracea* and *Arthrobacter* lipases are non-specific. An ER process using  
10 those lipases delivers a fully randomised fat, a fat randomised on all glyceride positions. However, those lipases either have been found to be not suited for upscaling to an industrial modification process and/or have not been approved for food manufacture.

15

The process described in EP 652289 uses a common 1,3-specific lipase and a substantial amount, at least 4 wt.% of diacylglycerides (also denoted as diglycerides). The resulting fat becomes fully randomised over all positions,  
20 but still contains the starting high amount of diglycerides and other byproducts which need to be removed by a subsequent purification process.

Recently, processes for enzymatic rearrangement have been  
25 reported where the above mentioned Lipozyme TL IM catalyst is employed: JAACS, Vol.78, no 1 (2001) pp. 57-64 and JAACS, Vol.79, no 6 (2002) pp. 561-565. In order to obtain 100% rearrangement relatively long enzyme contact times are needed. The corresponding low reactor throughput makes the  
30 process relatively expensive. Use of the process for randomising the fatty acid residues over all glyceride positions has not been considered and even is undesired because the process aims at selective 1,3-rearrangement.

A cost effective ER process which delivers a fat with a highly randomised middle position is badly needed.

5

#### STATEMENT OF THE INVENTION

A process has been found comprising the exposure of a triglyceride fat to a catalyst comprising a lipase resulting  
10 into a rearrangement of the fatty acid residues on the glyceride moiety over the terminal and middle positions, which rearrangement proceeds to a degree of conversion  $R_e$ , where  $R_e$  is selected from the range 0.3-0.95, the rearrangement comprising a randomisation of the fatty acid  
15 residues on the middle position to a degree of conversion  $R_a$ , where  $R_a$  is selected from the range 0.06-0.75, which process is characterized by the use of a *Thermomyces lanuginosa* lipase containing catalyst having an activity which is at least 250 IUN, while  $R_a > 0.32R_e - 0.08$ , the  
20 catalyst activity and  $R_a$  and  $R_e$  being as defined in the specification.

#### DETAILS OF THE INVENTION

25

The present process uses the *Thermomyces lanuginosa* lipase containing ER catalyst Lipozyme TL IM<sup>™</sup> which is commercially available from NOVOZYMES, Denmark as an aggregate of enzyme and silica and having varying activity values, including  
30 activities being at least 250 IUN. The method for establishing catalyst activity is described below. The invented process has the benefit over the prior art process that it is a one step process which does not need

undesirably high amounts of diglycerides for randomising the middle position of the glyceride.

Because moreover the degree of rearrangement Re is limited

5 to a maximum of 0.95 the process can be carried out with a high initial throughput in a packed bed reactor, using the short contact times known from W096/14756 which makes the process cost effective.

In the first hour that the reaction is run the residence  
10 time of the oil in the reactor is preferably <25 min, more preferably <20 min, still more preferably <15 min.

Residence time in hours is determined by dividing the volume of the catalyst bed by the volume of the oil passing the bed  
15 in one hour.

In contrast to the 1500 kg oil throughput per hour per 400 kg catalyst in a packed bed reactor, which is recommended by the supplier of the above catalyst, the present invention allows the processing of 4400 kg oil per hour with the same  
20 amount of enzyme. This corresponds with 32 minutes, and 11 minutes residence time respectively.

Initial throughput is based on fresh enzyme and obviously throughput is reduced to compensate for gradual loss of  
25 enzyme activity.

Equally, when using a batch process, cost effectiveness may be enhanced by a considerable reduction of the catalyst concentration which in the present process may be lower than the 10 wt.% of the above mentioned prior art processes. The  
30 catalyst concentration is selected from the range 0.05 - 9 wt.%, preferably 0.05 - 5 wt.%, more preferably 0.05 - 3 wt.% calculated on the reaction mixture.

Critical for the effect of the invention is that the catalyst activity exceeds the specified minimum value being 250 IUN.

5 In contrast to what is expected from a low water and a low diglyceride concentration, randomisation of the middle position of the glyceride molecule proceeds to a surprisingly high conversion degree in the present process. The effect is that even before a 100% rearrangement degree  
10 of the terminal positions has been attained, the degree of middle position randomisation is substantial. This is expressed in a high ratio  $R_a/R_e$  which is expressed by the equation  $R_a > 0.32R_e - 0.08$ , preferably  $R_a > 0.32R_e - 0.06$  more preferably  $R_a > 0.32R_e - 0.04$ , where  $R_a$  is the  
15 conversion degree of the middle position randomisation and  $R_e$  is the conversion degree of overall rearrangement.

For acting properly the Lipozyme TL IM catalyst does not need addition of water to the reaction mixture. The present  
20 process is carried out with a reaction mixture in which the content of water is low, preferably being in the range 0.001 - 0.1 wt.%, more preferably being in the range 0.001 - 0.05 wt.%. Measurement of the water content is done not earlier than 30 minutes after mixing the catalyst with the feedstock  
25 in a batch reactor. When a packed bed reactor is used, measurement is done in a sample of the oil leaving the reactor but not earlier than 30 minutes after startup of the reactor.

30 Water content is determined by means of Karl Fischer titration in a reaction sample from the reaction product which was allowed to stabilise for 30 minutes.

In the art of enzymatic rearrangement the presence of silica is reported to catalyze randomisation of the middle

5 position. However, when using a process according to the prior art but using the highly active TL IM lipase according to the present invention, it was after a decrease of the concentration of silica containing catalyst that a surprising increase of middle position randomisation was  
10 noticed.

Determination of the degree of conversion for the reaction,  $R_e$ , is established as follows:

15 For any starting fat blend, the feedstock, and for an actual reaction product the overall fatty acids composition, the composition of fatty acids on the middle position and the triglyceride composition is established using common analysis methods comprising FAME-analysis, the GLC/carbon  
20 number method and the HPLC/silver phase method as are described in for example EP 78568, EP 652289, JAOCS, (1991), 68(5), 289-293 and Hammond E.W.J., Chromatography, 203, 397, 1981.

25 Determination of the degree of conversion  $R_e$  for the enzymatically catalyzed reaction is based on the change of the carbon number profile. For a specific reaction product and its starting material the carbon number profile is determined by silverphase chromatography following the prior  
30 art methods as described above. The carbon number is the total number of carbon atoms in the three fatty acid residues of a triglyceride molecule.

The carbon number profile of a 1,3-specific fully randomized product is a theoretical value calculated from the measured triacylglyceride profile of the starting mixture.

Experimental information necessary to perform this

5 calculation can be obtained by analyzing the profile of fatty acid residues of the fat composition and by analysis of the profile of fatty acid residues situated at the 2-position of the triacylglyceride using the above mentioned methods.

10

The molar fraction of any specific fatty acid residue (pA) on either the sn-1 or the sn-3 position (which terminal positions of the glycerol backbone are assumed to be identical) is calculated according to the following formula:

15

$$pA_{sn1,3} = (3 * pA_{total} - 1 * pA_{sn2}) / 2$$

The indices "total" and "Sn2" relate respectively to the molar fraction of the fatty acid in the total fatty acid  
20 residue mixture of the fat and the molar fraction on the middle position of the glycerol backbone.

For all occurring fatty acid residues having a molar fraction larger than 0.002 the value  $pA_{sn1,3}$  is established.  
25 The distribution of these molar fractions should be normalized to 1.0.

Subsequently, the triacylglyceride profile for 100% 1,3-selective randomness is calculated by simple statistics  
30 known to the man skilled in the art which is based on the concept that a triacylglycerol e.g. of the type ABB occurs in a mole fraction  $p(ABB)$ :

$$p(ABB) = 2 * pA_{sn1,3} * pB_{sn2} * pB_{sn1,3}$$

From the resulting triacylglycerol profile the carbon number  
5 profile can easily be derived.

The degree of conversion is calculated according to the  
following scheme:

For each carbon number in the range 30 - 60 the differences  
10 are calculated between the mole fraction at the begin of the  
reaction and at the 100% 1,3-selective randomisation state.  
The sum of the absolute values of these differences defines  
the 100% absolute change of carbon numbers which points to  
the 100% conversion state.

15

For a specific sample taken from the proceeding reaction in  
the same way for each carbon number between 30 to 60 the  
differences between the fraction at the begin of the  
reaction and the fraction at the actual state of the  
20 reaction product are calculated. Again the sum of the  
absolute value of these differences is taken. This number is  
the actual absolute change of carbon numbers.

The degree of actual conversion  $Re = (\text{actual absolute change}$   
25  $\text{of carbon numbers}) / (\text{100\% absolute change of carbon}$   
 $\text{numbers})$ .

This equation applies for triglyceride products where the  
above 100% absolute change of carbon numbers is at least  
30 0.15. If not, the degree of conversion is determined in an  
alternative way:



The mole fractions are established for each triacylglyceride of the type H3, H2O and H2L, where H indicates fatty acid residues of palmitic or stearic acid, O of oleic acid and L of linoleic acid. H3 denotes a triacylglyceride containing 3 H type fatty acid residues and so forth for H2O and H2L. For each of these triglycerides the absolute change between the molar fraction at the begin of the reaction and at the 100% 1,3-selective random state is calculated. The sum of the absolute values of these changes defines the 100% degree of conversion, the 100% absolute change of triacylglyceride groups.

For a specific sample of the proceeding reaction the difference between the mole fraction at the begin of the reaction and at the actual state of the reaction product is calculated for the same triglycerides. The sum of the absolute value of these changes is taken. This number is the actual absolute change of triacylglyceride groups.

For the specific sample the degree of conversion  $R_e$  follows from the equation:

$$R_e = (\text{actual absolute change of triacylglyceride groups}) / (\text{100\% absolute change of triacylglyceride groups})$$

Determination of  $R_a$ , the degree of randomisation at the middle position:

The degree of randomness  $R_a$  is calculated from the profile of fatty acid residues of the total fat composition and the profile of fatty acid residues situated at the 2-position of the triacylglyceride. For establishing these data generally

GLC-FAME and 2-position analysis of the initial feedstock and of the reaction mixture is used according to the methods in the references described above.

5 For each fatty acid occurring in the triacylglyceride fat at a molar level of at least 0.002 the absolute difference of its Sn2 molar fraction at the begin of the reaction and its molar fraction in the total triacylglyceride fat is calculated. The sum of the absolute values of these changes  
10 for all fatty acids defines the status of 100% degree of randomness, denoted as Fa-Sn2-100%.

For a sample of the proceeding reaction for each of the same fatty acids in the same way the absolute change between the  
15 Sn2 molar fraction at the begin of the reaction and at the actual state of the reaction product is calculated. Again the sum of the absolute values of these changes is taken, which number is the actual absolute change of fatty acids on the Sn-2 position, denoted as Fa-Sn2-actual.

20

For the specific sample the degree of randomness  $R_a$  follows from the equation:  $R_a = (Fa-Sn2-actual) / (Fa-Sn2-100\%)$

25 The temperature of the reaction mixture preferably is selected from the ranges 40-85°C, preferably 45-80°C, more preferably 50-75°C.

The process of the invention can be applied on all kinds of  
30 triglyceride fat blends, but is most appropriate for triglyceride fat blends in which there is an imbalance in the distribution of fatty acids over the three glyceride positions. Similarly to chemical interesterification the

present process is able to increase the content of triglycerides with a saturated middle position which is a much desired feature for improving fat qualities.

5 The process is particularly suitable for feedstocks containing

- any mixture of a liquid oil and a fully hydrogenated oil,
- any triglyceride fat which has not been subjected to hydrogenation, preferably

10 - a mixture of palm fat or a palm fat fraction and a lauric fat or a lauric fat fraction.

The invention also comprises the use of an aggregate of *Thermomyces lanuginosa* lipase and silica for partially  
15 rearranging fatty acid residues of a triglyceride fat to a degree of 0.3-0.95, comprising a rearrangement on the middle position to a degree of 0.06-0.75, which lipase/silica aggregate has an activity of at least 250 IUN, preferably at least 300 IUN, more preferably at least 350 IUN.

20

An economy related benefit of the present process is that it does not need a cumbersome final purification from diglycerides as is necessary in the process of EP 652289.

25 The invention also comprises enzymatically rearranged triglyceride fats of which the degree of rearrangement  $Re$  is selected from the range 0.3-0.95 and of which the degree of glyceride middle position randomisation  $Ra$  is selected from the range 0.06-0.75, while  $Ra > 0.32Re - 0.08$ , preferably  $Ra$   
30  $> 0.32Re - 0.06$ , more preferably  $Ra > 0.32Re - 0.04$ , where the parameters  $Re$  and  $Ra$  are as defined elsewhere in this specification.

Fats according to the invention are suitable for the preparation of food compositions, particularly for the preparation of a constituting fat phase which comprises a liquid oil and a structuring fat. Such fat phases are widely

5 used for the preparation of fat continuous emulsions used in the manufacture of e.g. spreads.

Because an enzymatic rearrangement process qualifies as natural, those fats too may be qualified as natural.

10 The present process allows the production of fats enriched with triglycerides which have a saturated fatty acid residue on the middle position. This is an important feature for subsequent processing of such fat in food applications where the crystallisation behaviour of the lipid phase is critical  
15 for end product quality. Those triacylglycerides strongly influence both stability of the end products against adverse temperature conditions and crystallisation related process parameters based on the intrinsic crystallisation properties of the fat.

20 So the present process allows the production of the high palmitic fats which according to EP 831711 have a low graininess risk. This means that when the feedstock is a blend with palm oil or a palm oil fraction, the rearranged fat phase does not cause graininess in fat continuous  
25 emulsion spreads prepared with such fat phase, or at least the graininess risk is substantially reduced.

The invention also comprises the food products in which a  
30 fat is incorporated which is obtained by the process of the present invention.

The activity value of the used ER catalyst is measured as follows:

## 5 ESTABLISHING THE ACTIVITY OF THE LIPOZYME TL IM CATALYST

### 1. PRINCIPLE

10 The method is provided by the catalyst supplier and is based on interesterification of triglycerides by an immobilized lipase. The conversion of tristearin in a substrate composed of 27 w/w % fully hydrogenated soy bean oil and 73 w/w % refined, bleached and deodorized soy bean oil at 70 °C and 15 200 rpm stirring is used to quantify the activity of the catalyst. The concentration of tristearin is determined using an HPLC based analytical method.

20

### 2. SPECIFICITY AND SENSITIVITY

Components having the same retention time as tristearin in the chromatographic method used will cause an overestimation 25 of the tristearin concentration.

### 3. DEFINITION OF UNITS

30

The interesterification activity is defined as the initial conversion rate for tristearin at standard conditions. 1 IUN corresponds to a conversion rate of 0.01 g tristearin/1 /minute/ gram catalyst.

35

### 4. APPARATUS

Analytical balance

Shaker water bath

Pipettes

Positive displacement pipette for taking oil samples (due to high viscosity of the liquid).

- 5 Standard pipettes (e.g. Finnpiquette) for the other steps in the analysis.

10 HPLC HPLC system including the following modules: HPLC pump, injection system and column heater.

Light scattering detector, e.g. Sedex 55.

HPLC column Column RP 18e (5µm), LiChroCart 250-4.

- 15 Data acquisition Data can be acquired and processed using chromatography software.

#### ANALYTICAL CONDITIONS

20

Column temp.: 40°C

Flow: 1.5 mL/min.

- 25 Mobile phase: The mobile phase consists of 50 v/v % acetonitrile, HPLC grade and 50 v/v % dichloromethane, HPLC grade.

Injection volume: 20 µL.

30

Run time: At least 11 minutes.

Column temperature: 40°C (±2°C)

- 35 Detector: 40°C (±2°C), nitrogen pressure 2.3 bar.

Detector sensitivity: Gain 6

40

#### 5. REAGENTS AND SUBSTRATES

##### CHEMICALS

- 45 Tristearin. SIGMA grade, Approx. 99%

##### SOLUTIONS

Substrate (20 grams):

27 w/w % fully hydrogenated soy bean oil (delivered by ADM, Illinois, USA )

5 73 w/w % refined, bleached and deodorized soy bean oil  
(delivered by ADM, Illinois, USA)

Catalyst (2 gram): The samples are conditioned at  $a_w = 0.3$   
for at least 24 hours.

10

## 6. SAMPLES AND STANDARDS

### 15 STANDARDS

A standard curve of tristearin is made in the concentration  
range from 0.25 to 2.0 mg/ml.

### LEVEL CONTROL

20 Reference sample: Lipozyme TL IM reference PPW6503-3,  
particle size fraction 425-500 $\mu$ m, is used (single  
determination).

## 7. PROCEDURE

25

As substrate a blend of 27 w/w % fully hydrogenated soy bean  
oil (FH SBO) and 73 w/w % refined, bleached and deodorized  
soy bean oil (RBD SBO) is made. The oil mixture is heated to  
80 °C in a water bath, and is well mixed.

30

The oil mixture is weighed out in 100 ml conical flask with  
a screw top wail, about 20 grams in each. The precise weight  
is notated with 2 decimals. The flasks (batches) are placed  
in the shaker water bath at 70°C and 200 rpm.

A catalyst amount corresponding to approx. 10% of the amount of oil is weighted out ( approx. 2 grams). The precise weight of the catalyst is determined with 2

5 decimals.

When the oil mixture is homogeneous, a time zero-sample is taken. 100µl of the oil is taken with a positive displacement pipette (Gilson microman). The pipette tip is 10 wiped off by a Kleenex to remove outside oil mixture and the sample is deposited in a HPLC vial (type BROWN 12x32mm with Silicone/PTFE Septa).

The weighed amount of catalyst is added to the flasks 15 containing the oil mixture and samples are taken to the times: 15, 30, 45 and 60 minutes. All samples are 100 µl. The samples are stored in a freezer until HPLC analysis.

The samples in the HPLC vials are diluted with 900 µl 20 dichloromethane. This solution is mixed on a whirl-mixer, before a further dilution of 100 µl to 900 µl dichloromethane is made (total dilution of 100x). After a further mixing this sample is being analysed by HPLC.

25 The diluted samples are analysed by HPLC-ELSD.

The response versus concentration of the tristearin standards are fitted to an exponential model.

30

## 8. CALCULATION



The concentration of tristearin in the samples is calculated by use of the standard curve.

The conversion of (the decrease in) tristearin concentration

5 versus time is fitted to a exponential model, by non linear parameter estimation. The model is:

$$C_{Tristearin}(t) = C_{0,est} \cdot \exp(-k_{est} \cdot t)$$

10

Where

$C_{Tristearin}(t)$  = is the concentration of tristearin in the reaction mixture at time, t.

15  $C_{0,est}$  = start concentration of tristearin (estimated parameter)

$k_{est}$  = rate constant (estimated parameter)

t = reaction time

20 From the estimated rate constant,  $k_{est}$ , the activity at standard conditions, the IUN activity, can be calculated according to the following formula:

$$IUN/g = k_{est} \cdot C_{0,std} \cdot 100 \cdot \frac{W_{std}}{W} \cdot \frac{M_{oil}}{M_{oil,std}}$$

25

$C_{0,std}$  = start concentration of tristearin at standard condition.

$W_{std}$  = weight of catalyst under standard conditions (2g)

W = actual weight of catalyst

30  $M_{oil}$  = actual weight of oil

$M_{oil,std}$  = weight of oil under standard conditions (20g)

**Example 1**

5 60 g of palm oil and 40 g of palm kernel oil were mixed together in a 100 ml reaction vessel and heated to 70°C. 1 wt.% of Lipozyme TL IM (activity 455 IUN) was added to the mixture and this was stirred at 70°C for 10 hours. Samples 10 were taken at intervals and the degree of conversion Re and the degree of randomisation Ra were determined according to the methods described elsewhere in this specification by means of establishing changes in carbon number distribution and changes in composition of fatty acids on the middle 15 position.

At the conversion degrees Re of 0.85 and 0.5, the following degrees of randomisation of the middle position have been found:

TABLE I

20

Degree of Conversion Re	Degree of randomisation Ra
0.85	0.28
0.5	0.1

**Example 2**

Example 1 has been repeated but using a low acticity TL IM 25 lipase catalyst and the 10% catalyst concentration as used in the prior art.

**Example 3**

Example 1 has been repeated using the same high activity TL 30 IM catalyst, but with the same high catalyst concentration as used in comparison example 2, allowing a contact time which is much shorter than necessary in examples 1 and 2.

TABLE II

ER using TL IM catalyst	Example 1	Example 2	Example 3
Catalyst conc. (wt.%)	1	10	10
Water (wt.%)	0.013	0.025	0.024
Reaction time (h)	5.25	2.75	0.60
Activity (IUN)	455	160	455
Conversion Re	0.85	0.85	0.85
Randomness Ra	0.28	0.08	0.21

5 The high activity TL IM catalyst (example 1) causes at a low concentration the same conversion Re as the prior art low activity catalyst of example 2 but accompanied by a high randomisation of the middle composition. When increasing the catalyst concentration according to example 3 the same  
 10 effect as example 1 is obtained with a shorter contact time, which enables a higher throughput.

#### Examples 4 - 6

15 Examples 1 - 3 have been repeated under the same conditions, respectively, but using shorter contact times. The shorter contact times result in a lower degree of conversion, but even at these lower conversion degrees the same relatively high randomisation on the middle position is observed.

20

TABLE III

	Example 4	Example 5	Example 6
Conversion Re	0.4	0.4	0.4
Randomness Ra	0.08	0.02	0.08

## CLAIMS

(102)

1. Process comprising the exposure of a triglyceride fat to a catalyst comprising a lipase which process results into a rearrangement of the fatty acid residues on the glyceride moiety over the terminal and middle positions, which rearrangement proceeds to a degree of conversion  $R_e$ , where  $R_e$  is selected from the range 0.3-0.95, the rearrangement comprising a randomisation of the fatty acid residues on the middle position to a degree of conversion  $R_a$ , where  $R_a$  is selected from the range 0.06-0.75, which process is characterized by the use of a *Thermomyces lanuginosa* lipase containing catalyst having an activity which is at least 250 IUN, while  $R_a$  is greater than  $0.32R_e - 0.08$ , the catalyst activity and  $R_a$  and  $R_e$  being as defined in the specification.
2. Process according to claim 1, characterised in that the catalyst has an activity of at least 300 IUN, more preferably at least 350 IUN.
3. Process according to claims 1 or 2, characterised in that  $R_a$  is greater than  $0.32R_e - 0.06$ , preferably greater than  $0.32R_e - 0.04$ .

4. Process according to anyone of claims 1 - 3, characterised in that the amount of catalyst when used in a batch reactor is 0.05 - 9 wt.%, preferably 0.05 - 5 wt.%, more preferably 0.05 - 3 wt.% calculated on the reaction mixture.
5. Process according to anyone of claims 1 - 4, characterised in that in the first hour that the reaction is run in a packed bed reactor the residence time of the oil in the reactor is <25 min, preferably <20 min, more preferably <15 min.
6. Process according to anyone of claims 1 - 5, characterised in that the triglyceride fat is selected from the list consisting of
- any mixture of a liquid oil and a fully hydrogenated oil,
  - any triglyceride fat which has not been subjected to hydrogenation, preferably
  - a mixture of palm fat or a palm fat fraction and a lauric fat or a lauric fat fraction.
7. Process according to anyone of claims 1 - 6, characterised in that the rearrangement degree  $Re$  is less than 0.9, preferably less than 0.85.

8. Process according to anyone of claims 1 - 7, characterised in that the rearrangement degree  $Re$  is at least 0.35, preferably at least 0.4.

9. Process according to anyone of claims 1 - 8, characterised in that the content of water in the reaction product is selected from the range 0.001 - 0.1 wt.%, preferably from the range 0.001 - 0.05 wt.%.

10. Process according anyone of claims 1 - 9, characterised in that the temperature of the reaction mixture is selected from the range 40-85°C, preferably from the range 45-80°C, more preferably from the range 50-75°C.

11. An enzymatically rearranged triglyceride fat of which the degree of rearrangement  $Re$  is selected from the range 0.3-0.95 and of which the degree of glyceride middle position randomisation  $Ra$  is selected from the range 0.06-0.75, while  $Ra$  is greater than  $0.32Re - 0.08$ , preferably greater than  $0.32Re - 0.06$ , more preferably greater than  $0.32Re - 0.04$ , where the parameters  $Re$  and  $Ra$  are as defined in the specification.

12. A triglyceride fat according to the preceding claim which is obtained by enzymatic rearrangement of a fat selected from the list consisting of
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- any mixture of a liquid oil and a fully hydrogenated oil,
  - any triglyceride fat which has not been subjected to hydrogenation, preferably
  - a mixture of palm fat or a palm fat fraction and a lauric fat or a lauric fat fraction.
13. A triglyceride fat according to claim 11 or 12, characterised in that the rearrangement degree  $Re$  is less than 0.9, preferably less than 0.85.
14. A triglyceride fat according to anyone of claims 11 - 13, characterised in that the rearrangement degree  $Re$  is at least 0.35, preferably at least 0.4.
15. Use of an aggregate of *Thermomyces lanuginosa* lipase and silica for partially rearranging fatty acid residues of a triglyceride fat to a degree of 0.3-0.95, comprising a rearrangement on the middle position to a degree of 0.06-0.75, which lipase/silica aggregate has an activity of at least 250 IUN, preferably at least 300 IUN, more preferably at least 350 IUN.

16. A food products which comprises a fat according to anyone of claims 11 - 14.
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## ABSTRACT

Process comprising the exposure of a triglyceride fat to a catalyst comprising a lipase which process results into a rearrangement of the fatty acid residues on the glyceride moiety over the terminal and middle positions, which rearrangement proceeds to a degree of conversion  $Re$ , where  $Re$  is selected from the range 0.3-0.95, the rearrangement comprising a randomisation of the fatty acid residues on the middle position to a degree of conversion  $Ra$ , where  $Ra$  is selected from the range 0.06-0.75, which process is characterized by the use of a *Thermomyces lanuginosa* lipase containing catalyst having an activity which is at least 250 IUN, while  $Ra$  is greater than  $0.32Re - 0.08$ .

